# Handbook of Specimen Collection and Handling in Microbiology

Second Edition

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February 1981
Revised March 1983
Revised January 1985



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PUBLIC HEALTH SERVICE
CENTERS FOR DISEASE CONTROL
ATLANTA, GEORGIA 30333

# PREFACE

This handbook was designed to be used by laboratory and other medical personnel responsible for collecting and transporting specimens to the bacteriology laboratory. The handbook should also be of value to personnel responsible for hospital epidemiology and nosocomial infection control. The microbiology laboratory plays a critical role in successful patient care, but the value of its reports is dependent upon the first step in specimen handling—that is, selecting, collecting, and transporting the specimen to the bacteriology laboratory. In the final analysis, the clinical bacteriology laboratory can be of little value to the physician and thus offer only minimal service to patient care if specimens are improperly collected and submitted for the isolation and identification of microorganisms. Microbiologists must be aware also that misleading or insignificant information reported to a physician can be as harmful as incorrect results.

Laboratory policy should be formulated WITH THE PATIENT IN MIND. Laboratorians recognize that many patients cannot be expected to do exactly what is asked of them. The specimens received may be less than optimal but should not be accepted if the specimen is obviously inappropriate or meets one of the criteria for rejection. The guidelines suggested herein are not meant to be inflexible. The very nature of both patient and organism variability necessitates intelligent decisions and appropriate measures to provide significant information to the clinician; what may be "normal flora" in a "normal" individual may be a potential pathogen in an immunocompromised host.

This handbook is a compendium of facts arranged in outline and tabular form and lists basic principles of specimen handling along with the rationale supporting each principle. Use the following pages as guidelines only. It might be further suggested that the principles and procedures outlined in this handbook should be presented and discussed with the infection control committee or other hospital committees to elicit feedback for any changes or additions that must be made to satisfy local practice needs. Once the document has been approved by the appropriate committees, it should become part of the laboratory policy manual for the hospital. Hospital size should never be a factor in whether or not proper specimen collection and handling are carried out. If microbiology reports are a part of patient care, the procedures used to provide those reports must guarantee that the information given to the physician is, indeed, significant.

Portions of this handbook were taken from an excellent "Teledialog" series by Elmer W. Koneman, M.D., published by the Colorado Association for Continuing Medical Laboratory Education.[1] Other sections were taken from a three-part series of the Centers for Disease Control (CDC) Laboratory Updates (CDC 80-94; CDC 80-96) written by the author. These and other CDC Laboratory Updates are available from State Public Health Laboratories.

J. Michael Miller, Ph.D.

## How to Start With Clinical Microbiology Specimens

The microbiologist, in order to interpret specimen results properly, must have a basic knowledge of host-parasite relationships and must know how to correlate specimen results with the diagnostic needs of the patient. To accomplish the job of interpreting and correlating results accurately, many questions must be constantly addressed; some are listed below as examples:

1. Has the specimen which is submitted for culture been properly collected or obtained?
2. Has it been properly delivered to the laboratory?
3. Is the request clearly understood?
4. Does the necessary minimal patient information and history accompany the request?
5. Has the specimen been taken from a site that is normally sterile?
6. If the site has a normal flora, what is it?
7. To what extent may member(s) of this normal flora play a role in the pathogenesis of disease?
8. Is identification of any normal flora necessary?
9. What bacteria are most likely to cause disease when found in the site?
10. Is quantitation of suspected pathogens a significant adjunct to diagnosis?
11. How far does the microbiologist go with primary plating?
12. How far does the microbiologist go after a pathogen is isolated and presumptively identified?
13. What does the microbiologist do when a pathogen is not isolated?
14. Are antibiotic susceptibility tests routinely required?
15. Should the microbiologist in a clinical laboratory have a public health conscience?
16. Does the microbiologist know what his/her limitations and those of the laboratory are?

— *Taken from a lecture by*
**Albert Balows, Ph.D.**
Assistant Director for Laboratory Science
Center for Infectious Diseases, CDC

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# SPECIMEN SELECTION AND COLLECTION

*If a physician is dependent upon microbiology laboratory data for helping to save his patient, the ONE WHO COLLECTS THE SPECIMEN may determine the course of the patient's recovery.*

## SPECIMEN SELECTION AND COLLECTION

### General Considerations

1. Collect before antibiotic therapy whenever possible.
2. Collect material from where the suspected organism will most likely be found.
3. Observe asepsis in collection of all specimens.
4. Consider stage of disease.
5. Instruct patients clearly.
6. Use proper containers and/or transport media.
7. Deliver specimen promptly.
8. Provide sufficient information to the laboratory.

***Open the lines of communication with all persons potentially involved in the specimen handling process.***

## SECTION I: SPECIMEN SELECTION, COLLECTION, AND RATIONALE

### A. Requisition

The laboratory requisition ("lab slip") should include the following information:

1. Patient name
2. Patient age and sex
3. Patient room number or address
4. Physician name and address (or place physician can be located)
5. Specific anatomic culture site
6. Date and hour of specimen collection
7. Clinical diagnosis, special culture request, relevant patient history
8. Special procedures used in obtaining specimen
9. Name of individual transcribing orders
10. Antimicrobials, if any, patient is receiving

*The requisition form should provide as much information as needed for correct interpretation of laboratory results. The need for the patient's name and location is obvious. The patient's age may be important in certain instances; e.g., if special culture techniques are required or special pathogens considered. The physician's name and location is essential so that interim reports can be given. The exact anatomical culture site, clinical diagnosis, and special collection procedures used are essential for the microbiologist in selecting appropriate culture media. The name of person transcribing orders is needed should problems concerning the culture request arise.*

### B. Label

Each specimen should have a label firmly attached **to the specimen container** bearing the following information:

PATIENT NAME_____

HOSPITAL NO._____

ROOM NO._____

PHYSICIAN_____

CULTURE SITE_____

DATE_____ HOUR_____

Specimens requiring emergency (STAT) handling or which may contain a pathogen of potential danger (*Mycobacterium tuberculosis*, hepatitis virus, etc.) should be appropriately marked.

*Unfortunately, many specimen containers are received in the laboratory without labels or with labels that are not properly completed. All entries on the label* **must** *be legibly* **printed.** *Patient's first and last names should be used to prevent mixup of specimens from individuals with the same surnames. The hospital number or other designator is a valuable cross-check on the name. The patient's room number or address must be clearly indicated in the event re-collection of the specimen is necessary. The* **specific** *culture site should be indicated both to validate the specimen and to aid in media selection. The date and hour of collection should be indicated so that culture results can be properly interpreted.*

C. Collection Times

1. The optimal times for specimen collection must be based upon both the type of infectious disease process and the ability of the laboratory to process samples. Laboratories are usually better staffed during daytime hours to receive specimens.

   *The microbiology laboratory may not be well staffed during evening and late night hours. Samples collected late in the evening often do not produce adequate growth by the next morning. However, provisions must be made to handle urgent samples during "off" hours, and consultation with supervisory personnel is highly recommended.*

2. Twenty-four-hour specimen collections for culture should be discouraged and accepted only after consultation with the microbiologist or pathologist.

   *Pathogens in highest concentration in first morning collections will be diluted by added secretions. There is a high likelihood that samples stored after collection may become overgrown with contaminants. Improved laboratory extraction techniques preclude the need for large volumes of samples.*

3. The first early morning sputum and urine samples are optimal for recovery of acid-fast bacteria, fungi, and other pathogens. Samples collected at other times are acceptable.

   *Early morning secretions are more concentrated and more likely to contain large numbers of the etiologic agent.*

4. The timing of blood cultures* should be determined by the clinical condition of the patient. Physicians should always indicate the collection schedule. Except in acute cases of septicemia, blood cultures should not be drawn more frequently than 1/2 h apart. A total of three cultures per 24 h is usually sufficient to diagnose most cases of septicemia.

*A "blood culture" is defined as a draw of at least 10 ml of blood divided between two 50- to 100ml bottles–one incubated aerobically and one anaerobically. In children, 1 to 5 ml of blood is acceptable.

*In endocarditis, typhoid fever, brucellosis and other uncontrolled infections, the bacteremia is continuous, thus making timing of collection less critical. In other infections, bacteremia is intermittent and may precede the onset of fever by an hour, making collection timing important. In acute febrile episodes, two draws of at least 10 ml blood each, obtained from separate venipuncture sites, will allow immediate initiation of therapy. Samples drawn within 1/2 h may reflect the same bacteremic episode and sequential positive cultures may not be as valid as those spaced at longer time intervals. The recovery rate after three negative cultures per 24 h is extremely low, except in cases where a sudden fever spike is observed; then, drawing of an additional blood culture may be indicated.*

5. The following specimens should be collected only after consultation with the pathologist or microbiology supervisor and, if tested, the protocol should be published in the standard procedures manual:

   a. Viral cultures, unless the tests are done routinely
   b. Blood for serum-killing power tests or antibiotic drug assays
   c. Darkfield examinations for spirochetes or other bacteria
   d. Special blood cultures for recovery of fungi
   e. Recovery of chlamydia, rickettsia, leptospira, or other unusual organisms

   *These situations often require the use of special laboratory equipment and selection of enriched or selective media. Samples often must be collected at specific times or in special ways in order to ensure optimal recovery of microorganisms, or to produce results which can be interpreted in relation to therapeutic regimes. Physicians must bear the responsibility of informing the laboratory that an unusual infectious disease is suspected. If the physician or someone in the ward is to obtain the specimen, laboratory personnel should be consulted to determine the need for any special techniques or collecting devices.*

## D. Collection Procedures

1. All specimens must be collected in appropriate sterile containers. If samples are to be delayed in processing or are sent to reference laboratories, a transport medium must be used.

   *If the container is not sterile, results may be erroneous. It is the laboratory responsibility to see that sterile containers of suitable, leakproof construction are made available to physicians or ward personnel. Containers for stool cultures should be clean and leakproof but need not be sterile.*

2. Anaerobic cultures are best collected by aspirating abscess fluid with a sterile syringe and needle. Syringes can be capped with the needle holder and submitted for culture, or the aspirated fluid can be injected into an anaerobic transport vial. The submission of swabs for anaerobic culture is discouraged, but if swabs must be used, they should be placed immediately into gassed tubes or suitable anaerobic packets.

   *It is important to protect species of anaerobic bacteria from the killing effect of atmospheric oxygen and dessication. The chance for recovery is enhanced by protecting the specimen from any contact with atmospheric oxygen before inoculation in the laboratory.*

3. Sputum samples must contain lower respiratory secretions. Patients must be instructed to cough deeply. The mouth should be rinsed with water or gargle, and dentures should be removed immediately before the sample is collected.

   *All sputum samples are contaminated to varying degrees with oropharyngeal secretions. Mechanical rinsing of the mouth immediately before expectoration will reduce the number of contaminating bacteria. Induced specimens or transtracheal aspirations are recommended for adult patients who cannot produce sputum.*

4. Bronchial washings should be processed as soon as possible after they are collected. Currently, there is no documentation to support the use of an enrichment medium for delayed transport of such specimens for isolation of *M. tuberculosis.*

   *Some microorganisms which may infect the respiratory tract, such as* **Haemophilus influenzae,** *are susceptible to drying or low temperatures.* **M. tuberculosis** *specimens should be mailed "unenriched" to a reference laboratory. An in-transit decontaminating solution* developed at CDC has not been tested with bronchial washings but has successfully preserved* **M. tuberculosis** *in sputum specimens for days while killing most contaminating organisms.*

5. The collection of clean-catch urine samples must not be left to chance. Ideally, the specimen should be collected by a nurse or aide, or by the patient after specific instructions from a nurse or aide.

   *There is a high potential for contamination of the periurethral area in females from vaginal or bowel flora. Since most laboratories perform routine colony counts on all urine samples, meticulous care must be taken in specimen collection if valid results representative of bladder urine are to be obtained.*

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*cetylpyridinium chloride-sodium chloride (J. Clin. Microbiol. 1:411-413. 1975)

*If patients are to collect specimens unattended, specific verbal and written instructions will help to ensure collection of a good specimen. It may be well to actually read the instructions to the patient, particularly if there is a language barrier. It is recommended that these instructions be printed on a card for the patient to retain during the collection procedure. Instructions should be available in the predominant languages of the area. The requisition must indicate if the patient is symptomatic or asymptomatic.*

6. Stool specimens submitted for the recovery of acid-fast bacilli should not be processed.

   *It is virtually impossible to recover acid-fast bacilli from fecal material because of the inability to prevent heavy overgrowth with bowel flora*[1].

7. Surface lesions (wounds) must be sampled carefully. It is imperative that the surface lesion be opened and the advancing edge of the lesion firmly sampled. Pus must be expressed onto swab. Surface lesions are unsuitable for anaerobic studies.

   *Pus, alone, may not reveal growth upon plating since the encased organisms may be dead. The REPRESENTATIVE specimen is at the advancing margin of the wound. Never submit a dry swab that has been carelessly rubbed over a surface lesion. Anaerobes are abundant on skin surfaces and are common surface wound contaminants. Scrub the area around the wound carefully before sampling.*

8. Wound specimens submitted for anaerobic workup must be submitted in an appropriate anaerobic transport medium or in the syringe used to collect an aspirate.

   *Anaerobic transport media are designed to protect the strictest anaerobe. Other methods of transport may preserve some anaerobes for a time but may not allow optimal recovery of anaerobes. The physician's need for complete anaerobic data is no less than that of the laboratory for a properly selected and submitted specimen in anaerobic transport.*

9. Descriptive terms such as "wound," "eye," "genital," or other nonspecific terms are not as helpful to the laboratory as are *specific anatomic locations* describing the source of specimens along with a diagnosis.

10. Microbiology laboratories should subscribe to the complete Cumitech series published by the American Society for Microbiology, 1913 I Street, N.W., Washington, DC 20006.

## Specimens and Potential Pathogens Associated With Certain Diseases

| Specimen Selection | Potential Pathogens |
|---|---|
| **Bacterial Meningitis** | |
| 1. Spinal fluid<br>2. Blood<br>3. Wounds, etc.<br>4. Subdurals (infant)<br>5. Respiratory tract | 1. Adults, neurosurgical patients<br>a. *Neisseria meningitidis*<br>b. *Streptococcus pneumoniae*<br>c. *Staphylococcus aureus*<br>d. Others<br>2. Infants and children<br>a. *Escherichia coli*<br>b. Streptococcus - Group B<br>c. *Haemophilus influenzae*<br>d. *Neisseria meningitidis* |
| **Bacterial Eye Infections** | |
| 1. Purulent discharge<br>2. Lower cul-de-sac<br>or<br>3. Inner canthus | 1. *Haemophilus influenzae,* biotype III *(H. aegyptius)*<br>2. *Staphylococcus aureus*<br>3. *Moraxella* sp.<br>4. *Streptococcus pneumoniae*<br>5. *Streptococcus* sp.<br>6. *Neisseria gonorrhoeae*<br>7. *Pseudomonas aeruginosa*<br>(Reported STAT) |
| **Bacterial Otitis Media*** | |
| 1. Acute<br>a. No culture<br>b. Tympanic membrane aspirate<br>2. Chronic<br>Drainage | 1. Acute<br>a. *Streptococcus pneumoniae*<br>b. *Haemophilus influenzae*<br>c. *Streptococcus pyogenes*<br>d. Others<br>2. Chronic<br>a. *Pseudomonas aeruginosa*<br>b. *Proteus* sp.<br>c. Fungi (Aspergillus)<br>d. Anaerobes |
| **Bacterial Sinusitis*** | |
| *Nose and nasopharyngeal cultures are not predictive of the etiologic agent of bacterial otitis media or sinusitis. Aspirates are the specimens of choice. | 1. Acute<br>a. *Streptococcus pneumoniae*<br>b. *Streptococcus* sp.<br>c. *Staphylococcus* sp.<br>d. *Haemophilus* sp.<br>e. *Klebsiella* sp. and other Enterobacteriaceae |

**Specimens and Potential Pathogens Associated With Certain Diseases *(continued)***

| Specimen Selection | Potential Pathogens |
|---|---|
| | 2. Chronic<br>a. As above<br>b. Anaerobes<br>c. Fungi |
| **Wounds, Abscesses**<br>1. Purulent drainage<br>2. Tissue affected<br>3. Body fluids<br>4. Ulcers<br>5. Wound margins | 1. *Staphylococcus aureus*<br>2. Anaerobes (deep wounds, aspirates only)<br>3. Enterobacteriaceae<br>4. *Streptococcus* sp.<br>5. *Clostridium* sp.<br>6. Enterococcus<br>7. *Pseudomonas aeruginosa* |
| **Bacterial Throat-Pharynx Infections**<br>1. Pharynx & both fauces<br>2. Nasopharyngeal swab recommended for possible pertussis instead of "cough plate." Must use Regan-Lowe medium or Bordet-Gengou plates & streak immediately | 1. *Streptococcus pyogenes* or other beta-hemolytic *Streptococcus*<br>2. *Haemophilus influenzae*<br>3. *Corynebacterium diphtheriae*<br>4. *Neisseria gonorrhoeae*<br>5. *Bordetella pertussis* |
| **Bacterial Pulmonary Infections**<br>1. Transtracheal aspirate<br>2. Lung aspirate/biopsy<br>3. Bronchoscopy (?)<br>4. Sputum (?)<br>5. Blood | 1. *Streptococcus pneumoniae*<br>2. *Haemophilus* sp.<br>3. *Staphylococcus* sp.<br>4. *Klebsiella* sp.<br>5. Other Enterobacteriaceae<br>6. *Mycobacterium* sp.<br>7. Almost any organism in pure culture or heavy growth may be worked up and reported. |

**Specimens and Potential Pathogens Associated With Certain Diseases *(continued)***

| Specimen Selection | Potential Pathogens |
|---|---|
| **Possible Septicemia** | |
| Blood | 1. *Staphylococcus* sp.<br>2. *Escherichia coli*<br>3. *Klebsiella* sp.<br>4. *Pseudomonas* sp.<br>5. *Bacteroides* sp.<br>6. Enterococcus<br>7. *Streptococcus pneumoniae*<br>8. Other *Enterobacteriaceae*<br>9. *Candida* |
| **Bacterial Endocarditis** | |
| Cultures:<br>1. 2 or 3 blood cultures on first day<br>2. Repeat next day if initial cultures negative<br>3. Interval 1-6 h | 1. Viridans group *Streptococcus*<br>2. Enterococcus<br>3. *Staphylococcus* sp.<br>4. Enterobacteriaceae<br>5. Anaerobes |
| **Bacterial Diarrhea** | |
| 1. Stool<br>2. Rectal mucous *(Shigella)*<br>3. Blood (?) | *1. *Salmonella* sp.<br>*2. *Shigella* sp.<br>*3. *Campylobacter jejuni* (special media required)<br>4. *Yersinia enterocolitica*<br>5. *Vibrio* sp.<br>6. *Escherichia coli* (enterotoxigenic, enteroinvasive)<br>7. *Aeromonas/Plesiomonas* |
| **Genital Tract** | |
| 1. Cervix<br>2. Urethral discharge<br>3. Rectum<br>4. Lesions<br>5. (Darkfield)<br>Vaginal swabs are seldom helpful and should be discouraged. | 1. *Neisseria gonorrhoeae*<br>2. *Treponema pallidum*<br>3. *Haemophilus ducreyi*<br>4. *Trichomonas vaginalis*<br>5. *Candida* sp.<br>6. T-Mycoplasma<br>7. *Chlamydia trachomatis*<br>8. *Gardnerella vaginalis* (?)<br>9. Group B *Streptococcus* - Ob/Gyn<br>10. *Listeria monocytogenes* - Ob/Gyn |

*minimum for routine culture

**Specimens and Potential Pathogens Associated With Certain Diseases *(continued)***

| Specimen Selection | Potential Pathogens |
|---|---|
| **Urinary Tract Infections** | |
| 1. Clean catch midstream | 1. *Escherichia coli* |
| 2. Suprapubic aspirate | 2. *Klebsiella* sp. |
| 3. Catheterization | 3. *Proteus mirabilis* |
| 4. Infants - bag (?) | 4. *Pseudomonas* sp. |
| Catheter tips unacceptable for culture. | 5. Enterococcus |
| | 6. *Staphylococcus saprophyticus* |
| | 7. Others |
| **Bacterial Bone & Joint Infections** | |
| 1. Bone | 1. *Staphylococcus* sp. |
| 2. Joint aspirate | 2. *Haemophilus influenzae* |
| 3. Overriding skin lesions | 3. *Streptococcus* sp. |
| | 4. *Salmonella* sp. |
| | 5. *Neisseria gonorrhoeae* |
| | 6. Enterobacteriaceae |
| | 7. *Streptococcus pneumoniae* |
| | 8. *Pseudomonas* sp. |
| **Skin Infections** | |
| 1. Impetiginous lesions | 1. *Staphylococcus aureus* |
| 2. Cellulitis margins | 2. *Streptococcus* sp. |
| 3. Petechial lesions | 3. *Neisseria meningitidis* |
| 4. Bullae | 4. Enterobacteriaceae |
| 5. Pustules | 5. Dermatophytes and other mycotic agents |
| 6. Ulcers | 6. *Treponema pallidum* |
| | 7. *Mycobacterium marinum* |
| **Burn Infections** | |
| 1. Tissue | 1. *Staphylococcus* sp. |
| 2. Beneath eschar | 2. *Streptococcus* sp. |
| 3. Blood | 3. *Pseudomonas aeruginosa* |
| | 4. Enterobacteriaceae |
| | 5. *Candida* sp. |

**Specimens and Potential Pathogens Associated With Certain Diseases *(continued)***

| Specimen Selection | Potential Pathogens |
|---|---|
| **Newborn Systemic Infection** | |
| 1. Blood | 1. Enterobacteriaceae - *Escherichia coli, Klebsiella* sp. Others |
| 2. Spinal fluid | |
| 3. Urine | |
| 4. Respiratory tract | 2. *Staphylococcus aureus* |
| 5. Skin-umbilicus | 3. Streptococcus - Groups A,B,D |
| 6. Skin-ear | 4. *Haemophilus* sp. |
| 7. Consider: wounds, eye, etc. | 5. *Listeria monocytogenes* |
| | 6. *Streptococcus pneumoniae* |
| | 7. Others[1] |

**Suitability of Various Clinical Materials for Anaerobic Culture Studies**

| Suitable | Unsuitable |
|---|---|
| 1. Properly collected abscess material | 1. Throat or nasopharyngeal swab samples |
| 2. Blood | 2. Sputum, tracheostomy aspirate, bronchoscopic washings |
| 3. Bone marrow | 3. Voided or bladder catherization urine samples |
| 4. Lung aspirate and transtrachael aspirate | 4. Vaginal or cervical swabs |
| 5. Suprapubic urine aspirate | 5. Material from superficial abscesses or lesions improperly collected |
| 6. Endometrial or endocervical material collected by direct visualization through a speculum | 6. Specimens contaminated with feces (draining fistulae, colostomy, bowel contents, rectal abscesses) |
| 7. Aseptically collected tissue | 7. Feces* or rectal swab samples |
| 8. "Sulfur granules" from sputum or other materials when actinomycosis is suspected | |
| 9. Body fluids (ascitic, cerebrospinal, pericardial, pleural, synovial) | |
| 10. Bile | |

*There are a few exceptions; for example when botulism (especially infant botulism), *C. perfringens* foodborne disease, or antibiotic associated pseudomembranous colitis are suspected, it is appropriate to test stool specimens.

## Blood Culture Collection[2]

| Clinical Condition | Collection Protocol | Comments |
|---|---|---|
| **ADULTS and ADOLESCENTS** | | |
| Severe septicemia, meningitis, osteomyelitis, arthritis, pneumonia | Two cultures* prior to therapy | One 10- to 20-ml sample from each arm. |
| Subacute bacterial endocarditis | Three cultures within 24 h | Space each collection at least 1 h apart. Two should be collected at beginning of fever spikes. |
| Acute bacterial endocarditis | Three cultures within 1-2 h before therapy | |
| Low-grade intravascular infection | Three cultures within 24 h | Specimens collected at least 1 h apart. Two should be collected at first sign of febrile episodes. |
| Bacteremia of unknown origin – patient on therapy | Four to six cultures within 48 h | Take specimen just before next dose of antimicrobial agent. |
| Febrile episodes | No more than three total cultures | Bacteremia may precede episodes of fever and chills by about 1 h. |
| **YOUNGER CHILDREN** | | |
| | 1- to 2-ml samples | Two cultures usually suffice for diagnosing bacteremia in the newborn. |

*A "blood culture" is defined as a draw of at least a 10-ml sample of blood (except in small children) divided between two 50- to 100-ml bottles.

When collecting the initial blood culture, consider collecting one tube of blood as an acute-phase serum for tests which may be needed in later studies with a convalescent-phase serum.

## Environmental Sampling

| Recommended for Routine Sampling | Frequency of Sampling | Acceptable Result | Comment |
|---|---|---|---|
| Sterilizers | | | Spore test every load if it contains implantable objects |
| Steam | Weekly (or monthly)* | No growth | *Bacillus stearothermophilus* spores |
| Hot air | Monthly (or weekly) | No growth | *Bacillus subtilis* spores |
| Gas | Weekly (or monthly) | No growth | *Bacillus subtilis* spores - test every load if implantable object included |
| Infant formulas manufactured in the hospital | Weekly | Less than 25 organisms/ml | Commercially prepared formulae need not receive an in-house check unless sterile bulk formula is transferred to individual bottles |
| High-level *disinfected* items (not sterilized)<br>Anesthesia equipment<br>Respiratory therapy apparatus<br>Endoscopes | At least quarterly; more often if previous results dictate | No organisms grown<br>No viable vegetative pathogens | |
| Water used to prepare Dialysis fluid | At least monthly | Less than 200 organisms/ml | |
| Dialysate during use | At least monthly | Less than 2000 organisms/ml | |
| **Laminar flow hoods in pharmacy | Optional (perhaps) | No growth or rarely growth on open plates. | Compounded materials should not be tested; open blood agar plates left at back of hood for 1 h during operation |
| **Laminar flow hoods (routine checks with "DOP" smoke) | Semiannually or when hood is moved or obvious accident | As per manufacturers' specifications | Filters, air-flow, etc. should be on regularly scheduled, preventive-maintenance program |

*Federal laboratory standards require at least monthly testing with spore strips.
**Not included in current (1981) CDC Guidelines for Prevention and Control of Nosocomial Infections.

# SECTION II
# SPECIMEN TRANSPORT

*Microorganisms are living things—rapidly they grow, they reproduce, they die. Transport media are designed to prevent or slow all three processes. Incomplete or misleading laboratory data may result if* any *of the three occur before the specimen can be cultured in the laboratory. Please hurry; the work can't be started until the specimen arrives!*

## SECTION II: SPECIMEN TRANSPORT AND RATIONALE

### A. Specimen Transport

1. It is important that culture specimens be processed as soon as possible after collection, preferably within 2 h. If longer delays are unavoidable, a suitable transport medium must be used. If urine samples will be delayed, they should either be refrigerated, inoculated to primary isolation medium before transport, or transported in preservative solution. Agar paddle devices may be used at the discretion of the laboratory.

   *Many species of bacteria are vulnerable to delays in processing, temperature changes, and decreased moisture; during prolonged transport, rapidly growing bacteria may overgrow more fastidious pathogens. Colony counts on urine samples are not valid if not processed within 1 h of receipt because of rapid doubling time of many urinary tract pathogens. If the urine is not cultured within 1 h, refrigerate the specimen. Refrigerated transport or use of an acceptable urine preservative is recommended if the specimen is to be sent by a private office to a private laboratory.*

2. If a delay in transport is anticipated, or if cultures are sent to a reference laboratory, Stuart's, Amies, or Carey & Blair transport medium should be used. Dry swabs are unacceptable.

   *Transport medium is formulated to maintain the viability of bacteria but allow only a slow rate of replication. Fastidious strains, however, may not survive the nutritionally poor medium. Some bacterial populations may double within 1 h if body fluids are present.*

3. When possible, specimens should be delivered directly to the microbiology laboratory, bypassing central collection areas or other departments in the laboratory.

   *We are not measuring chemicals, enzyme levels, or body cells, but living, replicating organisms that cannot be expected to conform to our schedules of convenience, no matter how busy we may be.*

4. See topic of "Specimen Refrigeration."

#### Transport Media

**Stuart's (1954)**

1. Originally formulated for transport of *Neisseria gonorrhoeae.*
2. Used charcoal-impregnated swabs which caused difficulty in Gram stain interpretation.*
3. "Non-nutritive" medium.
4. Good for most specimens.
5. Some gram-negative rods can utilize glycerophosphate in the medium, thus overgrowing the culture.

**Amies (1965)**

1. Modified Stuart's medium.
2. Replaced glycerophosphate with a balanced salt solution.
3. Retained charcoal but incorporated it into the medium rather than the swab.*
4. Better transport system for most specimens.

**Cary & Blair (1964)**

1. Similar to Stuart's but modified for fecal specimens.
2. pH increased from 7.4 to 8.4.
3. Removed charcoal from formula.
4. Good for stool specimens.
5. Recommended for fecal specimens suspicious for *Campylobacter* sp. and for other enteric pathogens.

**Buffered glycerol saline**

1. Designed for stool specimens only.
2. Good for mailing fecal specimens.
3. High pH to favor fecal pathogens.
4. Not for transport of fecal specimens in which *Campylobacter* sp. is suspected.

*"Modified" formulas may not include charcoal.

## Specimen Refrigeration

Specimens that **CAN** be refrigerated before inoculation of media:

- •Urines
- •Stools/Feces
- •Respiratory Exudates
- •Wounds

Specimens that **CANNOT** be refrigerated before inoculation of media:

- •Spinal Fluids and Other Body Fluids
- •Genital/Cervical for Gonococcus Isolation
- •Blood

If processing is delayed, **spinal fluids** should be held at 35°C.

**Environmentally Fragile Organisms**

| Organism | Most Likely Specimen | Comment |
|---|---|---|
| *Shigella* sp. | Stool | Immediate processing |
| *N. gonorrhoeae* | Genital | Sensitive to cold, needs 5%-10% $CO_2$ soon after collection |
| *N. meningitidis* | Cerebrospinal fluid (CSF) | Do not refrigerate; process soon after receipt in laboratory |
| *H. influenzae* | CSF, eye, ear, throat | Sensitive to cold |

## Collection and Transport of Clinical Specimens for Bacteriologic Examination

| Specimen | Collection Equipment | Transport | Instructions (comments) |
|---|---|---|---|
| Anaerobe-special request | Gassed-out tubes<br>Needle and syringe<br>Anaerobic swab transport device | No refrigeration needed<br>Use anaerobic transport method | 1. Avoid all $O_2$ exposure<br>2. Expel air from syringe<br>3. Label properly<br>4. Hold a needed supply of media in anaerobic atmosphere for better initial growth. Not critical |
| Blood | Commercial kit<br>Needle and syringe | Culture broth in bottles<br>50 ml/bottle. 5 ml blood/bottle. 2 bottles; one aerobic, one anaerobic. Do not refrigerate<br>100 ml/bottle with 10 ml blood/bottle. | 1. Decontaminate puncture site–(soap)-alcohol-iodine-alcohol<br>2. Do not palpate disinfected site<br>3. 10% (vol:vol) blood:broth<br>4. Decontaminate bottle stopper–iodine-alcohol |
| CSF | Surgical prep and collection by physician<br>Sterile screw-cap or snap-cap tubes | Transport in collection tube<br>Do not refrigerate | 1. Surgical prep of puncture site<br>2. Obtain "as much as possible": 4-5 ml is optimal for adults 0.5-1.0 ml in children<br>3. Handle as EMERGENCY specimen; hand carry to laboratory<br>4. One tube only, send to bacteriology first. Second and/or third, routinely to bacteriology |
| Ear | Aspirate from tympanocentesis (otitis media). Swab of drainage | Transport medium | 1. Clean external ear surface<br>2. CAREFULLY take representative area<br>3. Label properly |

**Collection and Transport of Clinical Specimens for Bacteriologic Examination** - *Continued*

| Specimen | Collection Equipment | Transport | Instructions (comments) |
|---|---|---|---|
| Eye | Swab (small) for each eye<br>Corneal scraping (by physician) | Transport medium | 1. Do not touch external skin<br>2. Obtain maximum material. Culture both eyes<br>3. Label properly<br>4. Prepare smears for Giemsa and/or Gram staining |
| Feces | Clean or sterile collection cup<br>Swab (only if necessary) | Refrigerate if not plated within 1 h<br>Swab into transport medium<br>Cary-Blair recommended for *Campylobacter* sp. | 1. Best specimen is diarrheal stool<br>2. Swab is satisfactory in acute cases but not for routine specimens or surveys<br>3. Insert swab beyond anal sphincter. Swab must show feces |
| Genital | Swab | Do not refrigerate<br>Immediate $CO_2$ for GC<br>Incubate at 35°C overnight before mailing | 1. Collect cervicals with a swab inserted through a speculum<br>2. Avoid touching swab to uninfected mucosal surfaces<br>3. Clean external urethra before taking urethral specimen<br>4. For GC, inoculate a modified Thayer-Martin plate at bedside, if possible<br>5. Prepare slide for staining using a second swab<br>6. Label properly |
| Nasopharynx | Cotton-tipped nichrome or stainless wire-28 ga | Do not refrigerate<br>Transport medium | 1. Nasal speculum helpful<br>2. Pass through nose into nasopharynx<br>3. Allow to remain for a few seconds<br>4. Carefully withdraw<br>5. Label properly |

**Collection and Transport of Clinical Specimens for Bacteriologic Examination** - *Continued*

| Specimen | Collection Equipment | Transport | Instructions (comments) |
|---|---|---|---|
| Nose | Swab | Transport medium | 1. Swab anterior nares only<br>2. Culture quickly |
| Sinus (tract)<br>(paranasal) | Curet or surgical specimen<br>Aspirate | Transport medium | 1. Insert and remove carefully<br>2. Prepare slide for stain using second swab or after inoculating media |
| Sputum | Sterile cup | Refrigerate if needed<br>Transport in collection container | 1. Carefully instruct patient to cough deeply (not to spit)<br>2. First morning specimen is best (no 24-h collection)<br>3. Transport immediately; seal container tightly<br>4. Consider sputum potentially contaminated with *M. tuberculosis* |
| Throat | Swab (tongue blade is necessary) | Transport medium if more than 2 h delay to laboratory | 1. USE TONGUE BLADE<br>2. Sample ONLY back of throat between & around the tonsillar area thoroughly<br>3. Avoid cheeks, teeth, etc.<br>4. Use silica gel packets to hold specimen more than 24 h |
| Urine (midstream) | Sterile screw-cap cup | Transport in collection container<br>Refrigerate quickly | 1. Give patient clear and detailed instructions<br>2. Clean with soap, not disinfectant<br>3. A l-h delay before culturing is too long<br>4. Refrigerate no longer than 24 h prior to culture<br>5. Seal container tightly |

## Collection and Transport of Clinical Specimens for Bacteriologic Examination - *Continued*

| Specimen | Collection Equipment | Transport | Instructions (comments) |
|---|---|---|---|
| Urine (catheter) | Sterile screw-cap tube<br>Needle and syringe | Sterile tube | 1. Collect from catheter line<br>2. Do not culture Foley tips<br>3. Decontaminate line as with venipuncture or use port |
| Wounds (surface) | Swab | Transport medium | 1. Decontaminate surrounding skin<br>2. Open lesion and express pus onto swab; sample advancing margin of lesion<br>3. Label properly |
| Wound (deep) | Syringe<br>Anaerobic swab kit | Anaerobic transport<br>Transport aspirate in the collecting syringe<br>or<br>Place aspirate into anaerobic transport container or vial<br>or<br>Collect pus onto swab and place directly into anaerobic transport (not recommended) | 1. Maintain anaerobic conditions<br>2. Label properly |

# SECTION III
# SPECIMEN PROCESSING

*A properly processed specimen provides only a certain amount of information. Interpreted properly, the generated data becomes useful. A physician's initial diagnosis is based upon observation of, and symptoms from, his patient. A microbiologist's interpretation of that patient's specimen results requires no less pertinent information.*

## SECTION III: SPECIMEN PROCESSING AND RATIONALE

### A. General

1. Specimens should not be processed until the requisition slip and the label are correctly prepared.

   *The person transcribing the orders of the doctor should be called by the laboratory to complete the requisition or verify questionable responses.*

2. Specimens should not be processed if received in inappropriate containers or improper transport medium, or if received after a prolonged delay. Call physician or ward nurse to see if second specimen can be conveniently obtained.

   *Insignificant information may mislead the attending physician. Outpatients or difficult collection procedures must be considered, then appropriate decision made based on a specific case. Report should clearly indicate specimen inadequacy and that results may not be valid or complete.*

3. The second specimen obtained from the same site within 24 h should not be processed unless there are specific orders by the physician.

   *Duplicate orders most commonly represent clerical errors which are costly to the patient and take up unnecessary laboratory time. There are few instances when two cultures within 24 h are clinically indicated.*

### B. Sputum Specimens

1. Sputum samples of less than 2 ml in volume should not be processed unless the material is obviously purulent.

   *With the exception of legionellosis, most respiratory bacterial infections cause copious amounts of sputum to be expectorated. Small quantities of a clear, thin material usually represent saliva.*

2. Only one sputum sample per 24 h should be submitted, except for postbronchoscopy specimens. If more than one specimen is received in series, the first morning specimen or the one with no microscopic evidence of contamination should be selected for processing.

   *One sputum sample per 24 h is usually adequate to reflect the respiratory secretion pool. Postbronchoscopy specimens usually represent the most ideal deep-cough specimens that can be obtained.*

3. A direct Gram stain should be performed on all routine sputum specimens to assess their quality as representative of lower respiratory secretions. A scoring system must be used (see p. 32).

   *Samples which are representative of "spit" rather than true lower respiratory secretions produce insignificant results. The reporting of a potentially pathogenic bacterium in a nonrepresentative sputum sample could be misleading, particularly in cases of clinical pneumonitis.*

4. Each smear is stained and several fields of view are examined and evaluated. Published criteria are available which describe methods to predict the absence of infection or significant contamination with oral secretions (see p. 32).

   *Squamous epithelial cells are derived from the oral mucosa and their presence in sputum samples represents contamination with "spit." Another specimen should be requested. If a contaminating organism from the oropharynx is incorrectly considered the pathogen causing the pneumonitis, therapy may be misdirected. For this reason, it is recommended that the Gram stain results be reported along with the culture results. Grading criteria are not applicable to sputum specimens submitted for isolation of* ***Mycobacterium tuberculosis*** *or mycotic agents.*

C. **Urine Specimens**

1. Colony counts should be performed routinely on all urine samples.

   *It is generally agreed that greater than 100,000 cfu/ml in clean-catch urine specimens are indicative of a urinary tract infection. There* are *instances when less than 100,000 cfu/ml are isolated from infected patients. In symptomatic patients, counts as low as 10,000 cfu/ml may be significant.*

2. Anaerobic cultures should not be set up on routine clean-catch urine specimens.

   *Urinary tract infections are rarely caused by anaerobic bacteria. If an anaerobic infection is suspected, a suprapubic bladder aspiration should be performed.*

3. Foley catheter tips should not be accepted for culture.

   *It is impossible to remove a catheter without contaminating it with microorganisms inhabiting the urethra.*

4. Urine samples collected from indwelling catheter bags should not be accepted for culture. If cultures are to be taken from indwelling catheters, a needle and syringe is used for urine aspiration through the rubber connector or the catheter line.

   *Stagnant urine in a catheter bag will be overgrown with bacteria, making culture results misleading and insignificant. More fastidious pathogens also will be overgrown with more rapidly growing coliforms.*

D. **Wound Specimens**

1. In order to determine if wound samples represent a superficial or deep specimen, direct Gram stains can be performed to evaluate the relative numbers of neutrophils and squamous epithelial cells.

   *Clinically infected wounds almost always produce a pyogenic reaction and many polys should be seen in Gram stains. The presence of abundant epithelial cells indicates a superficial sample or contamination from the skin of the wound margins.*

2. Anaerobic cultures should not be routinely set up on wound cultures which are not submitted in an appropriate anaerobic container.

   *Physicians should use clinical judgment when ordering anaerobic cultures. They are quite expensive and require considerable technical expertise. Gas production, foul odor, and copious pus production are clinical indications of anaerobic wound infections.*

## E. Spinal Fluids

1. Direct Gram stains should be performed on the centrifuged sediments of all spinal fluids submitted for culture. All positive results must be immediately called to the physician.

   *Immediate results may represent life-saving information in some cases of meningitis. Even a report of no bacteria may be important information in the assessment of clinical cases of meningitis. A simple methylene blue stain of spinal fluid also gives rapid information on the presence or absence of bacteria.*

2. If specimen processing is to be delayed, spinal fluids should be placed in a 35°C incubator until they can be inoculated to culture media. In no instance should samples be stored in the refrigerator.

   *Spinal fluid itself is a good innate culture medium. In most instances, infections are caused by one species and overgrowth with contaminants is not a concern. Organisms such as* ***Haemophilus influenzae*** *and* ***Neisseria meningitis****, common causes of meningitis, are sensitive to chilling and may die out in refrigerated samples.*

## F. Throat and Nasopharyngeal Cultures

1. Throat cultures should be routinely processed for the recovery of beta hemolytic streptococci only.

   *Organisms other than beta hemolytic streptococci do not cause primary acute pharyngitis. Staphylococci may cause tonsilar abscesses,* ***H. influenzae*** *constrictive epiglottitis and* ***Corynebacterium diphtheriae*** *will cause a membraneous pharyngitis.*

2. Attempts to recover routinely *H. influenzae* from throat cultures are the prerogative of each hospital or laboratory but should be discouraged.

   *A high percentage of healthy adults and children harbor* ***Haemophilus*** *species in their oropharynx. Physicians must inform the laboratory if* ***Haemophilus*** *infection is suspected.*

3. Antibiotic susceptibility testing should not be performed on bacterial isolates recovered from throat cultures.

   *Beta hemolytic streptococci are universally susceptible to penicillin. Other organisms are not considered pathogenic in the absence of specific complications.*

4. Coliform bacilli in throat cultures are not usually reported.
   *Coliform bacilli do not normally cause pharyngitis but can colonize the throat and serve as a reservoir for lower respiratory infections. Hospitalized patients tend to colonize with coliform organisms that are resistant to many antibiotics used in that hospital. Susceptibility tests should not be performed except on request or for nosocomial purposes.*

## G. Vaginal and Endometrial Cultures

1. In general, vaginal cultures are of minimal value and laboratories should resist processing them. Cultures for gonorrhea should be obtained directly from the uterine cervix. Anaerobic cultures should not be performed except on abscess fluid aspirated by syringe and needle from a paravaginal abscess. Other infections such as trichomonas, candidiasis, or those caused by *Gardnerella vaginalis* may be diagnosed by direct mounts, smears, or other tests.
   *The normal vaginal flora includes a wide variety of aerobic and anaerobic organisms. Anaerobic cultures set up on vaginal swab specimens are impossible to interpret in light of the normal background flora. Direct Gram stains of vaginal secretions for an assessment of bacterial flora, particularly in search for* ***Neisseria gonorrhoeae,*** *may be misleading because of the various resident organisms that can mimic or simulate organisms known to be pathogens. Physicians should be discouraged from submitting vaginal swabs for culture.*

2. Endometrial cultures should be collected by direct vision through the endocervical canal and placed in an anaerobic container. These cultures should always be processed for aerobic and anaerobic organisms.
   *Because the environment of the endometrial cavity is relatively anaerobic, it is not uncommon for anaerobic infections to take place. In cases of postpartum infection, organisms such as Group-B streptococci and* ***Listeria monocytogenes*** *should be specifically cultured. A double lumen catheter should be used to collect the specimen.*

## H. Miscellaneous Cultures

1. In processing of eye cultures, enriched chocolate agar should be used to detect fastidious organisms such as *Haemophilus* sp., *Neisseria* sp., or slow-growing gram-negative bacilli. A direct Gram stain should always be examined at the time cultures are set up.
   *Fastidious organisms often infect the eye and may be missed if an enriched culture medium is not used. The presence of gram-negative bacilli that morphologically resemble* ***Pseudomonas*** *species should be made immediately known to the physician because this organism can rapidly cause blinding ophthalmitis.*

2. Tissue biopsy specimens should be processed only after mincing and grinding the sample with a sterile pestle and mortar. Anaerobic cultures should be set up on request or if gas or a foul odor is detected by the technologist. Direct Gram stains should be performed on the ground eluate, and any positive findings should be reported immediately to the physician.

   *Anaerobic cultures may be valid only if the tissue has been submitted in a container protected from exposure to atmospheric oxygen although in larger specimens the reducing properties of the tissue proteins tend to maintain a relative anaerobic environment.* ***Clostridium perfringens*** *may be immediately detected by Gram stain. Other organisms such as staphylococci and streptococci also have characteristic morphology.*

3. Gastric specimens in general do not reveal meaningful results, except perhaps in septic infants or in older individuals with high intestinal obstruction. Bacterial colony counts on gastric secretions are of questionable value.

   *Anaerobic bacteria may inhabit the normal gastric secretions and interpretation of culture results may be difficult. The presence of large numbers of bacteria in gastric secretions usually indicates an alkaline pH shift from regurgitation of duodenal secretions in cases of intestinal obstruction. The recovery of certain species of mycobacteria may be significant. Gastric aspirates for mycobacteria should be neutralized before holding for processing.*

4. Body fluids, such as joint fluids, pleural fluids, and peritoneal fluids, should be inoculated to enriched media. Direct Gram stains may be very helpful in deriving a presumptive diagnosis and may also be helpful in selecting the proper culture media. It may be necessary to use an anticoagulent with some specimens.

   *Body fluid effusions and transudates often contain proteins and clotting factors which produce a clot or gel. Bacteria may be trapped in these clots and not appear in cultures of the fluid itself. It is recommended that body fluids be allowed to settle or undergo centrifugation in order to concentrate any bacteria present. This may not be possible if the specimen is allowed to clot. Enriched media should always be used since fluids often are infected with fastidious organisms in low numbers.*

5. Fecal specimens for *Yersinia enterocolitica*[3] should be inoculated to media routinely used for fecal pathogens. An additional MacConkey agar and CIN plate* should be inoculated and incubated at 25°C. The rectal swab or specimen should be placed in M/15 phosphate buffer and refrigerated at 4°C for "cold enrichment" of *Yersinia* sp. Plate to Mac & CIN at 7, 14, and 21 days and incubate the plates at 25°C.

   *The serotypes of* ***Y. enterocolitica*** *found in the U.S. (05 and 08 are most common) may be more easily isolated if cold enrichment is used. This organism is more often isolated from extraintestinal sources and, at present, is not a frequently isolated pathogen in the U.S. Procedures for its isolation should be available but the physician should be notified as to the length of time required before a negative report can be submitted. Alert the physician to the delay of a* **final** *report. Another potentially shorter regimen involves mixing equal volumes of feces and 0.5% KOH. Mix vigorously and plate to Mac and S-S. Final readings can be made in 48 h.*

*CIN agar (cefsulodin-irgasin-novobiocin) is a selective medium for *Yersinia*, is commercially available, and has been shown to be effective.

***Specimen Priority*** **(Three Classes)**[4]

| Urgent | Routine | Elective |
|---|---|---|

Specimens should be processed as soon as possible after they arrive in the laboratory.

***Urgent*** – *Specimens that represent potentially life-threatening illness requiring immediate attention so that some preliminary information may be forwarded to the submitting physician within 30 minutes to 1 hour of specimen arrival.*

Specimens with **urgent priority**:

Blood
Spinal fluid
Transtracheal aspirate
Eye (endophthalmitis)
Pericardial fluid
Amniotic fluid
} Regardless of location

All lower respiratory specimens – from intensive care units (ICU's)
Surgical – from ICU's
Joint fluid – if diagnosis of septic arthritis

***Routine*** – *Specimens submitted from patients at no immediate risk of life-threatening sequale but that represent a potentially important infectious event requiring diagnostic confirmation or preventive observation.*

Specimens with **routine priority**:

Throat, Pleural fluids, Burns, Eye .... Regardless of diagnosis or location

Urine (cath.) – if diagnosis of sepsis
Urine (voided or cath.) – from ICU
Female genital – Ob/Gyn or operating room in diagnosis of sepsis or septic abortion
Surgical – from operating room (OR)
Lower resp. specimens – if diagnosis of pneumonia
Peritoneal fluid – if diagnosis of pneumonitis

***Elective*** – *Specimens whose processing is handled as expertly and judiciously as routine specimens but whose results may be more of a confirmatory nature rather than an emergency diagnostic procedure.*

Specimens with **elective priority**:

All other specimens

**Specimen Priority for Processing**

| Specimen | Priority | Replica limits |
|---|---|---|
| Blood | Urgent | 6/2-day pd. |
| Body fluids (not cerebrospinal fluid [CSF]) | | none |
| Amniotic fluid | Urgent | |
| Pericardial fluid | Urgent | |
| Joint fluid (arthritis) | Urgent | |
| Bone marrow | Routine | |
| Peritoneal fluid | Routine | |
| Pleural fluid | Routine | |
| Ascitic fluid | Elective | |
| Bile | Elective | |
| Transudate | Elective | |
| Joint fluid (not arthritis) | Elective | |
| CSF | Urgent | none |
| Environmental Specimens | | |
| Intravenous fluid | Routine | see p. 13 |
| All others | Elective | |
| Eye | Routine | none |
| Genital, female (not anaerobe) | | 1/day/type |
| Endocervix, Vaginal, Urethra, Placenta, Vulva, Lochia, Perineum — Ob/Gyn | Routine | |
| Endocervix, Vaginal, Urethra, Placenta, Vulva, Lochia, Perineum — Sepsis/abortion | Routine | |
| Endocervix, Vaginal, Urethra, Placenta, Vulva, Lochia, Perineum — All other | Elective | |

## Specimen Priority for Processing (Continued)

| Specimen | | Priority | Replica limits |
|---|---|---|---|
| Genital, female (anaerobe) | | | 1/day/type |
| Placenta, C-section<br>Endometrium<br>Uterus<br>Culdocentesis<br>Fallopian tube<br>Cervical aspirate<br>Ovary<br>Bartholin's gland | | ICU's | Urgent | |
| | | Sepsis/abortion | Routine | |
| | | Ob/Gyn/OR | Routine | |
| | | All other locations & diagnosis | Elective | |
| Genital, male | | Elective | 1/day/type |
| Postmortem specimens | | Elective | |
| Lower Respiratory | | | |
| Tracheal<br>Bronchial<br>Sputum | | ICU's | Urgent | 1/day |
| | | Pneumonia | Routine | 1/day |
| | | All other locations & diagnosis | Elective | 1/day |
| Transtracheal aspirate | | Urgent | none |
| Lung biopsy | | Urgent | none |
| Upper Respiratory | | | 1/day/type |
| Throat | | Routine | |
| Nose | | Elective | |
| Oral | | Elective | |
| Ear | | Elective | |
| Sinus | | Elective | |
| Nasopharynx | | Elective | |
| Stool/Rectal | | Elective | 1/day |
| Surface specimens | | | 1/day/type |
| Burns | | Routine | |
| Cyst<br>Decubitis<br>Exudate<br>Laceration<br>Lesion<br>Paronychia<br>Skin<br>Stoma<br>Suture<br>Ulcer<br>Vesicle | | Elective | |

## Specimen Priority for Processing (Continued)

| Specimen | | Priority | Replica limits |
|---|---|---|---|
| Surgical Specimens | | | |
| Abscess | | | 1/day |
| Aspirate | | | 1/day |
| Biopsy | ICU's | Urgent | none |
| Bone | | | none |
| Clot/hematoma | OR | Routine | 1/day |
| Drain | | | 1/day |
| Exudate | All other locations | Elective | 1/day |
| Fistula | | | 1/day |
| IV catheter | | | 1/day |
| Prosthesis | | | none |
| Pus | | | 1/day |
| Stone | | | none |
| Tissue | | | none |
| Wound | | | 1/day |
| Urine | ICU's | Urgent | 1/day |
| | Other | Routine | 1/day |

## Criteria for Rejection[4,5,6]

| Problem | Action |
|---|---|
| 1. Unlabeled or improperly labeled specimen | 1. Telephone doctor or nurse. Have someone come to laboratory and identify specimen before it is processed. If no answer, put doctor on page. Repeat call after 24 h. Process specimen but do not publish results until doctor has been consulted. |
| 2. Prolonged transport<br>a. Urine -> 1 h at room temperature<br>b. Stools for trophozoites -> 1 h since collection<br>c. Gonorrhea specimens -> 1 h without transport medium | 2. Alert submitter of the discrepancy and request a repeat specimen. Not problem on report:<br>"Received after prolonged delay." |
| 3. Improper container (nonsterile) | 3. Do not process. Call submitter and request repeat specimen. If doctor insists on processing, call supervisor. |

## Criteria for Rejection[4,5,6] (Continued)

| Problem | Action |
|---|---|
| 4. Leaking container | 4. Do not process sputum, blood, viral specimens. Call submitter for repeat specimen and autoclave the leaking one. Other specimens - call submitter and ask for repeat specimen. Otherwise, note discrepancy on report. Protect laboratory personnel from possible infection. |
| 5. Oropharyngeal-contaminated sputum | 5. Do not report (or process). Indicate discrepancy in report. Request another specimen. |
| 6. Obvious foreign contamination | 6. Alert submitter of discrepancy. Request repeat specimen. |
| 7. Duplicate specimens submitted at the same time | 7. Select the one of best quality for culture. Report by note on report form. |
| 8. Duplicate specimens on *same day* for the *same* request (except blood) | 8. Place specimen in refrigerator. Call submitter and indicate duplicity. Culture only on request. |
| 9. Specimen unsuitable for culture request; i.e., anaerobe request from aerobic transport | 9. Call submitter, indicate discrepancy. Request proper specimen for the work requested. |
| 10. Quantity not sufficient (QNS) | 10. ***Blood*** - if less than 5 ml from adult, inform submitter and request another specimen. Process - note problem on report.<br>***Body fluids*** - if QNS for multiple requests, call doctor and determine priority of request. |

## Direct Examination by Gram Stain

A. Specimens received in the laboratory on which a direct Gram stain can be performed and results given to the physician.

1. Spinal fluid and other body fluids
2. Urine
3. Eye
4. Any purulent discharge
5. Sputum, transtracheal aspirate
6. Surgical aspirates
7. Tissue
8. Urethral exudates from males (for *N. gonorrhoeae*)

Report Gram morphology and exudate characteristics.

B. Disease states in which a direct Gram stain may prove helpful.

1. Meningitis
2. Brain, spinal, epidural abscess
3. Severe pneumonia
4. Endocarditis
5. Peritonitis
6. Gas gangrene
7. Necrotizing fasciitis
8. Potential postoperative sequelae of heart valve replacement, intra-abdominal infection, etc.
9. Gonorrhea (males)
10. Diphtheria
11. Vincent's angina

**Fecal Leucocytes in Stool Specimens From Patients With Diarrheal Disease[7]**

| Disease | Predominant Cell Type in Feces (Acute Illness) |
|---|---|
| Campylobacteriosis | Polymorphonuclear |
| Shigellosis | Polymorphonuclear |
| Salmonellosis | Polymorphonuclear |
| Typhoid fever | Mononuclear |
| Cholera | None |
| Enterotoxigenic *Escherichia coli* | None |
| Invasive *E. coli* colitis | Polymorphonuclear |

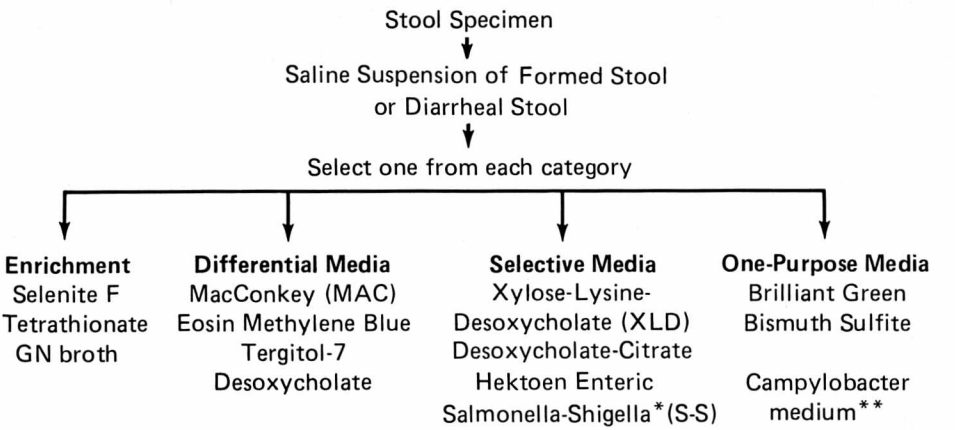
**Four Criteria for Grading Sputum Specimens**
*(Select one for your laboratory)*

The following criteria indicate oropharyngeal contamination and suggest that the sputum specimen may not be representative of the lower respiratory tract.

1. Greater than 10 squamous epithelial cells per 100X total magnification field[8].
2. Less than 25 polymorphonuclear leucocytes per 100X field[9].
3. Greater than 10 epithelial cells **and** less than 25 polymorphonuclear leucocytes per 100X field[10].
4. Greater than 25 squamous epithelial cells per 100X field[11].

Other methods as published by Bartlett[5] and Barry[12] assign a positive number to the quantity of white blood cells observed and a negative number to the quantity of epithelial cells present. By adding the positive and negative values gathered from a sputum smear, any negative sum would be a criterion for rejection.

**Handling Stool Specimens**




A **routine** protocol might include:
Selenite F
MAC
XLD
Campylobacter medium

*S-S agar is not recommended for isolation of Shigella since *S. sonnei* does not grow well on this medium.

**One-purpose media for the isolation of *Campylobacter jejuni* are commercially available. Select one to be routinely inoculated for each diarrheal stool specimen.

## GENITAL SPECIMENS

### Female Genital Specimens

| Not Cultured for Anaerobes | Cultured for Anaerobes |
|---|---|
| Endocervical | Placenta, C-section |
| Vaginal | Uterus (endometrial) |
| Urethra | Culdocentesis |
| Placenta | Fallopian tube |
| Vulva | Cervical aspirate |
| Female genital | Ovary |
| Lochia | Bartholin's gland |
| Perineum | |

### Male Genital Specimens

**Not Cultured for Anaerobes**

Urethral

Prostatic fluid

Seminal fluid

### Incubation Conditions

SPECIMENS TO BE INCUBATED UNDER 5%-10% $CO_2$

Genital – blood, chocolate, Modified Thayer-Martin.

Wounds, eye – blood, chocolate.

Respiratory (except throat) – blood, chocolate.

Blood – subculture chocolate and blood.

Body fluids – blood, chocolate.

SPECIMENS TO BE INCUBATED UNDER REDUCED $O_2$

Feces (for Campylobacter) – Campylobacter one purpose medium

5% $O_2$, 10% $CO_2$, 85% $N_2$ or

Packaged Campylobacter atmosphere

**Initial Processing and Examination of Eye Specimens**

| Organisms Seen on Gram Stain* | Growth on Solid Media | Growth in Broth | Action |
|---|---|---|---|
| yes | yes | yes | Isolate, identify, MIC test |
| yes | yes | no | Isolate, identify, MIC test |
| yes | no | yes | Isolate, identify, MIC test |
| yes | no | no | Rule out antibiotic treatment<br>Rule out anaerobes<br>Call report to physician |
| no | no | no | Report as "no growth" after 48 h |
| no | no | yes | Subculture broth – report pathogens only** |
| no | yes | no | Report pathogens only** |
| no | yes | yes | Report pathogens only** |

*Common contaminants may be seen as "rare" organisms on Gram stain, whereas true infections usually reveal many organisms per field in the presence of neutrophils.

**See Cumitech 13 for common pathogens necessary for workup.

## Culture Inoculation Guide for Eye Specimens[13]

| Clinical Entity | Source of Material | Cultures | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | BA | CA | 135C Thio | ANA | LJ | SAB | BHI |
| Cellulitis, preseptal | Abscess drainage | ✓ | ✓ | ✓ | ✓ | | | |
| Cellulitis, Acute | Abscess drainage | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ |
| Canaliculitis | Canalicular material | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ |
| Dacryocystitis, Acute | Conjunctiva | ✓ | ✓ | ✓ | | | | |
| | Drainage material | ✓ | ✓ | ✓ | | | ✓ | ✓ |
| Blepharitis, Acute | Lid margin | ✓ | ✓ | | | | | |
| Blepharitis, Chronic | Lid margin | ✓ | ✓ | | | | ✓ | |
| Conjunctivitis, Acute* | Conjunctiva | ✓ | ✓ | | | | | |
| " , Neonatal | Conjunctiva | ✓ | ✓ | T-M | | | | |
| Keratitis, suppurative | Conjunctiva | ✓ | ✓ | ✓ | | | ✓ | |
| | Cornea | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Endophthalmitis | Conjunctiva | ✓ | ✓ | ✓ | | | ✓ | |
| | Wound abscess | ✓ | ✓ | ✓ | | | ✓ | |
| | Fistula | | | | | | | ✓ |
| | Intraocular fluid | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

*Include culture or transport medium for chlamydia.

BA – 5% sheep blood agar
CA – Enriched chocolate agar
135C thio – supplemented thioglycolate
ANA – anaerobic blood agar plate
LJ – Lowenstein-Jensen medium
SAB – Saboraud dextrose plate
BHI – Brain heart infusion broth
T-M – Thayer-Martin

## Urine Culture Decisions

| Cultures With Pure or Mixed Isolates of: | Clean Catch Specimen From:* | | |
|---|---|---|---|
| | Asymptomatic Patient | Symptomatic Patient | Suprapubic Aspirates |
| Gram-negative rods | A | B | E |
| Enterococci | A | B | E |
| Coagulase-positive staphylococci | A | B | E |
| *Staphylococcus saprophyticus* | A | B | E |
| Commensals (*S. epidermidis*, *Neisseria* sp., diphtheroids, alpha- and nonhemolytic streptococci, *Lactobacillus* sp.) | D | C | C |

*Document symptomatic vs. asymptomatic patient. If unable to document, handle specimen as asymptomatic source.

A – Identify; antimicrobial susceptibility test (AST) when numbers exceed 100,000cfu/ml. If less than 100,000 cfu/ml, follow "C" (below) if mixed.

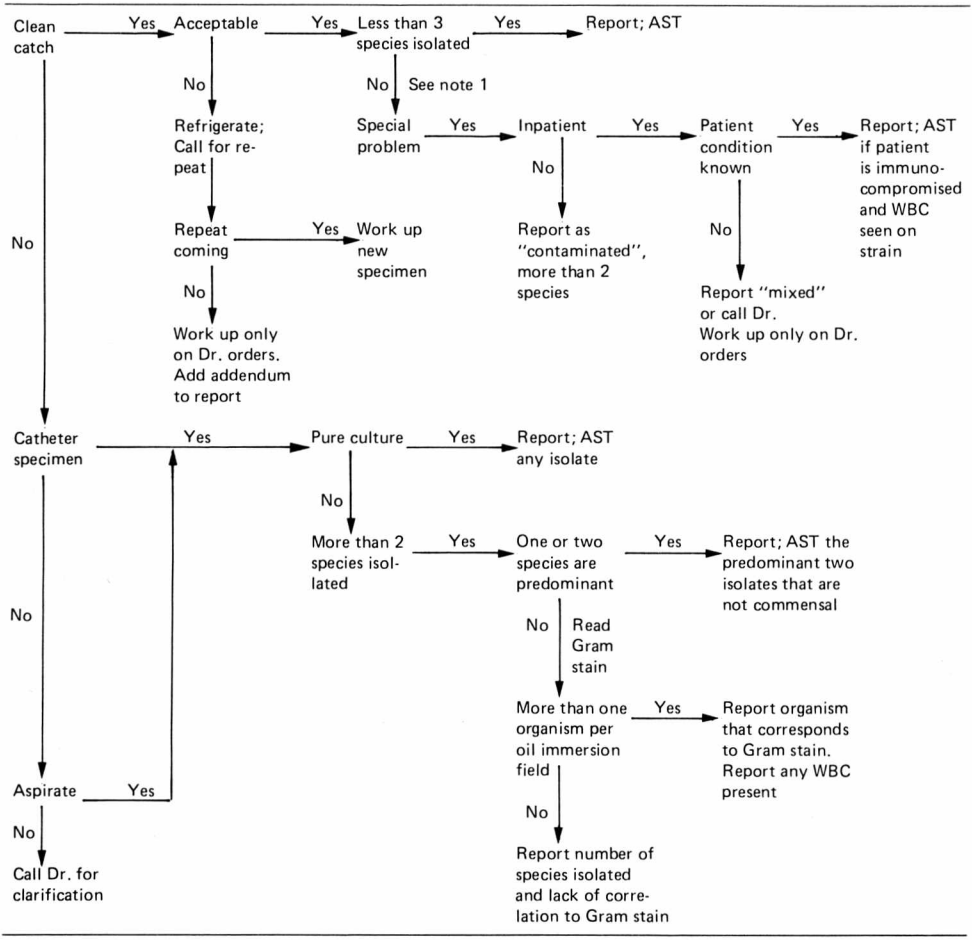
B – Identify; AST when numbers exceed 10,000 cfu/ml in the symptomatic patient.

C – Report as "(Mixed) commensal flora" and the number of commensal types.

D – Do not identify. Report number and type only.

E – Identify; AST any number isolated.

## Suggested Workup Diagram for Urine Cultures




Note 1: Report "More than two species isolated. Indicates contamination with commensal flora. Please repeat specimen." If patient is a special problem, follow the chart.

AST = antimicrobic susceptibility test; WBC = white blood cells

## Selected Examples of Media for Primary Isolation of Microorganisms

### General Purpose Media – Enriched

**Blood Agar (Sheep-5%)**
Enriched medium used for the primary recovery of most commonly encountered microorganisms.

**Chocolate Agar (Enriched)**
Hemolyzed blood agar with supplements that serves as an enriched medium for recovery of such fastidious organisms as *Neisseria gonorrhoeae* and *Haemophilus influenzae.*

**Blood Agar (Anaerobic)**
Nonselective blood agar used to recover anaerobic bacteria from clinical specimens. Contains sheep blood supplemented with yeast extract, hemin, Vitamin $K_1$, and L-cystine. Kanamycin and vancomycin may be added for selective purposes. May or may not be held anaerobically before inoculation.

### Enteric Media

**MacConkey Agar, Eosin Methylene Blue Agar, Tergitol 7, or Desoxycholate Agar**
Differential medium for the recovery of Gram-negative bacilli. Most Gram-positive bacteria are inhibited.

**Salmonella-Shigella (S-S) Agar, or Hektoen Enteric (HE) Agar or Xylose-Lysine-Desoxycholate (XLD) Agar, Desoxycholate-Citrate Agar**
Media more selective than MacConkey or Eosin Methylene Blue, designed specifically to recover species of Salmonella or Shigella from feces or other body fluids or secretions. S-S Agar is not recommended for the isolation of *S. sonnei* but is helpful for the isolation of *Y. enterocolitica.*

**Bismuth Sulfite (BS) or Brilliant Green (BG) Agar**
One-purpose media for the isolation of all *Salmonella* sp. including *S. typhi* (BS) or for *Salmonella* sp. other than *S. typhi* (BG).

**Gram-Negative (GN), Selenite Broth, or Tetrathionate Broth**
Enrichment broths for concentration of pathogenic Salmonella or Shigella from contaminated clinical specimens, particularly feces.

**CIN Agar**
One-purpose medium for the isolation of *Yersinia enterocolitica.*

### Inhibitory Media – Special Use

**Blood Agar (Phenylethyl Alcohol)**
Selective medium for the isolation of Gram-positive organisms, especially for specimens heavily contaminated with Gram-negative organisms, such as *Proteus* sp. which are inhibited by this medium.

**Modified Thayer-Martin Agar (MTM)**

A modified chocolate agar containing enrichments and the antibiotics vancomycin, colistin, and nystatin to inhibit growth of bacteria other than pathogenic *Neisseria* species. In the modified formula, trimethoprim lactate has been added and the agar concentration increased to 2% to inhibit the spreading of *Proteus* species. The carbohydrate concentration has also been increased to 0.25% to enhance growth. Other modifications are available.

### Broth Media

**Thioglycollate Broth (enriched with hemin and Vitamin K) or Chopped Meat Broth**

Designed for recovery of anaerobes from clinical materials.

**Trypticase Soy Broth or Tryptose Phosphate Broth**

General nutrient broths used for recovery of fastidious bacteria, for subculturing colonies of bacteria or for preparing the standard inoculum for the Bauer-Kirby susceptibility test.

### Transport Media

**Transport Medium: Stuart's, Amies, or Carey & Blair**

A transport medium should be available in all laboratories to maintain viability of microorganisms in specimens which must be forwarded to reference laboratories (see p. 15).

## Supplemental Primary Culture Media for Recovery of Special Fastidious Organisms

### Medium and Purpose

**Bordet-Gengou Potato Infusion Agar**

Selective medium for recovery of *Bordetella pertussis.* Inoculation of medium with a nasopharyngeal swab is recommended instead of the use of a cough plate.

**Brucella Agar**

Enriched peptic digest casein medium designed for recovery of Brucella species from clinical specimens and other infected material.

**Fletcher's Semisolid Medium**

Basal medium used with serum enrichments for the recovery of Leptospira species.

**Loeffler Medium and Tellurite Medium**

Used for inoculation of nasopharyngeal cultures for recovery of *Corynebacterium diphtheriae.* Also used to prepare methylene blue stains of suspicious colonies.

**Lowenstein-Jensen (LJ) Agar, Middlebrook 7H10 or 7H11 Agar**

Primary recovery of mycobacteria. Growth on LJ medium needed to demonstrate niacin accumulation. Middlebrook agar is transparent and used for microscopic examination of colonies.

**Mannitol Salt Agar**

Selective medium for the isolation of staphylococci.

**Peptone Water (Alkaline pH=8.4), Thiosulfate-Citrate-Bile salts-Sucrose Agar (TCBS)**

Primary recovery of *Vibrio cholerae* from fecal or other contaminated material.

**Regan-Lowe Medium**

Selective medium containing cephalexin in a charcoal agar base used for culture of *Bordetella pertussis.*

**Skirrow's, Campy BAP, Other Selective Media for Isolation of Campylobacter.**

Special media with a variety of antibiotics that are designed to inhibit normal fecal flora but not affect growth of *Campylobacter jejuni* when incubated at 42°C. All liquid stools should be routinely screened for *C. jejuni.*

| Type of Specimen | Media and Conditions | Normal Flora | Common Pathogens | Comments |
|---|---|---|---|---|
| Throat | Blood agar<br>35°C aerobic | Alpha and gamma strep<br>Commensal *Neisseria*<br>*Staphylococcus epidermidis*<br>Diphtheroids<br>*Streptococcus pneumoniae*<br>Anaerobes<br>*N. meningitidis* | Group A, beta-hemolytic streptococci<br>*(Corynebacterium diphtheriae)*<br>*(Bordetella pertussis)* | Chocolate agar should be used if *Haemophilus influenzae* is suspected. Should not be routine. The epiglottis should *not* be swabbed in suspected cases of epiglottitis. |
| Respiratory | Blood agar<br>Chocolate agar<br>35°C, $CO_2$<br>MacConkey (aerobic)<br><br><br>Anaerobic media..... | Larynx, trachea, sinus, Sputum:<br>*S. epidermidis*<br>Non-beta streptococci<br>Diphtheroids<br>Commensal *Neisseria*<br>Haemophilus<br><br>Transtracheal and Bronchial:<br>None | Group A, beta streptococci<br>*H. influenzae*<br>*S. aureus*<br>Enterobacteriaceae<br>*Pseudomonas* sp.<br>*S. pneumoniae* | Gram stain:<br>Sputum<br>All aspirates |
| Urine<br>Clean catch specimen colony count<br>Catheterized or aspirate | Blood agar<br>MacConkey<br>35°C, aerobic | None | *Escherichia coli* and other enterics<br>Enterococci<br>*Pseudomonas* sp.<br>Staphylococci | |

**Specimen Processing in Bacteriology** - *Continued*

| Type of Specimen | Media and Conditions | Normal Flora | Common Pathogens | Comments |
|---|---|---|---|---|
| Feces | MacConkey<br>Xylose-Lysine-Desoxycholate<br>Selenite F<br>35°C, aerobic<br>Selective medium for *Campylobacter*<br>42°C, 5% $O_2$ | Anaerobes<br>Enterobacteriaceae<br>Enterococci | *Salmonella* sp.<br>*Shigella* sp.<br>*Yersinia enterocolitica*<br>*Campylobacter jejuni*<br>*E. coli* (enterotoxigenic, enteroinvasive) | |
| Genital for GC only | Modified Thayer-Martin (MTM) 35°C, $CO_2$ | Lactobacilli<br>Diphtheroids<br>Alpha streptococci<br>Enterobacteriaceae<br>Enterococci<br>} inhibited on MTM | *Neisseria gonorrhoeae* | Gram stain |
| Vaginal, cervical, routine genital | Blood agar<br>Enriched chocolate<br>MTM<br>35°C, $CO_2$<br>MacConkey - aerobic | Lactobacilli<br>Diphtheroids<br>Enterococci<br>Enterobacteriaceae<br>Non-beta streptococci<br>Anaerobes<br>*G. vaginalis* | *N. gonorrhoeae*<br>*Candida albicans*<br>Group A and B streptococci<br>*Listeria monocytogenes* | Culture for *G. vaginalis* is of questionable value. |
| Genital – surgical or aspirates | Blood agar<br>Chocolate agar<br>MTM<br>35°C, $CO_2$<br>MacConkey - aerobic<br>Anaerobic blood agar | None | Same as above plus anaerobes | Gram stain |

## Specimen Processing in Bacteriology - *Continued*

| Type of Specimen | Media and Conditions | Normal Flora | Common Pathogens | Comments |
|---|---|---|---|---|
| Sterile body fluids<br>CSF, joint fluid,<br>pleural fluid,<br>peritoneal fluid,<br>etc. | Blood agar<br>Chocolate agar<br>35°C, $CO_2$<br>MacConkey agar<br>Thioglycolate<br>35°C, aerobic<br>Anaerobe blood agar<br>(except for CSF) | None | Identify all isolates<br>*S. pneumoniae*<br>*N. meningitidis*<br>*H. influenzae*<br>Gram-negative rods | Gram stain |
| Blood | Submitted in two bottles: one 35°C aerobic and one 35°C anaerobic | None | Any isolate potentially significant | |
| Wound<br>(superficial)<br>includes eye and ear | MacConkey agar<br>35°C, aerobic<br>Blood agar<br>Chocolate (eye)<br>35°C, $CO_2$ | *S. epidermidis*<br>Diphtheroids<br>Commensal *Neisseria*<br>Anaerobes<br>Other skin flora | *S. aureus*<br>Beta-hemolytic streptococci<br>*P. aeruginosa*<br>*H. influenzae* Biotype III<br>*(H. aegyptius)*<br>Enterobacteriaceae | Gram stain |
| Wound<br>(surgical or<br>aspirate)<br>Tissue specimens | Same as above plus<br>anaerobic blood agar<br>K-V blood agar*<br>Thioglycollate | None | Same as above plus<br>anaerobes<br>Potentially any isolate | Gram stain |

*Kanamycin-Vancomycin

## Alternative Avenues to Consider in Laboratory Diagnosis

### When should *Mycobacterium* sp. be considered?

1. With most all sputum specimens
2. When smears reveal poorly stained or diphtheroid-like organisms, but routine bacteriologic culture fails to grow anything within 48-72 h
3. In cases of cervical lymphadenitis
4. When cultures on routine bacteriology media fail to yield growth
5. When cultures in thioglycollate broth are still negative after several days of incubation
6. When the patient fails to respond to treatment with common antibacterial drugs
7. When serologic tests fail to reveal a rise in antibody titer to the suspected pathogen(s)

### When should fungal cultures be considered?

1. When spinal fluid with increased lymphocytes has a negative Gram stain and acid-fast stain
2. When a Gram stain of aspirated pus is negative
3. When sputum culture and Gram stain repeatedly fail to yield anything significant bacteriologically in a compromised host
4. When a Gram stain (and acid-fast stain, depending on tissue) of a surgical specimen is negative. Fungal cultures can be made of some specimens following examination of Gram stain
5. When blood cultures are negative for bacteria in a compromised host who is deteriorating for unknown reasons
6. When any mould or encapsulated yeast appears on blood agar
7. When the specimen is a skin biopsy of a granulomatous lesion

## Qualifying Statements to Accompany Certain Bacteriology Reports[5,6]

The microbiology laboratory is often requested to process and test specimens that are not obtained from the proper site, are of inadequate quantity, are improperly obtained, are submitted in inappropriate containers, or are submitted under other unacceptable conditions. When the laboratory is aware of any of the above conditions but is, nonetheless, required to process the specimen and submit results regardless of the relevance or irrelevance of the report, a qualifying statement at the end of the report (or as the only report) is desirable to convey the true value of the results.

Other occasions may arise in which the laboratory is requested to perform a test or report results when the action is neither warranted nor recommended. Again, a qualifying statement reflecting discretion and judgment should accompany the results to explain the potential source of error in the report or its interpretation.

The following are examples of qualifying statements that may be included with laboratory reports:

| Condition | Sample Addendum to Bacteriology Report |
|---|---|
| 1. *Direct specimen examination:* | Superficial material; no evidence of inflammation. Please consult microbiologist regarding further examination. |
| Many squamous epithelial cells; no leucocytes, particularly in:<br>eyes<br>wounds<br>genital specimens<br>respiratory specimens | Correlation with Gram stain suggests that this (these) isolate(s) may not relate to infection and may represent colonization or contamination.<br><br>Abundant squamous cells and few, or no, neutrophils suggest that this material is superficial and may contain contaminating or colonizing bacteria unrelated to infection. |
| 2. *Duplicate specimens* | Duplicate_____ specimens were received from this patient on the same day. The one of best quality was selected for culture. |
| 3. *Quantity not sufficient* (QNS) | Results may be misleading since the quantity of the specimen received was inadequate for proper processing. |
| 4. *Multiple isolates from certain specimens* | These isolates may or may not be true etiologic agents. Please submit the tentative diagnosis and/or list the specific anatomic site on the request slip. |

| Condition | Sample Addendum to Bacteriology Report |
|---|---|
| a. No patient information | The lack of information on the request slip prevents accurate differentiation of commensal *vs.* pathogenic flora. |
| b. > 3 anaerobes* | Mixed anaerobic flora. |
| c. > 5 total aerobes and anaerobes* | Mixed aerobic and anaerobic flora. |
| d. > 3 aerobes only* | Mixed _____ flora (skin)(fecal)(etc.) |
| 5. *Anaerobic request but aerobic transport* | Anaerobic studies were not performed because the specimen was submitted in an aerobic transport system. Strict anaerobes will not survive aerobic transport.<br><br>This report may be incomplete or misleading because the request for anaerobic studies was not accompanied by a specimen in anaerobic transport media. |
| 6. *Susceptibility test request on inappropriate organism* | Susceptibility testing is not warranted since multiple isolates were present with no predominating species.<br><br>Disk diffusion susceptibility testing on this (these) isolate(s) is not recommended since the test was designed only for rapidly growing aerobic organisms.<br><br>This (these) organism(s) is (are) not normally tested by the disk diffusion procedure. The reported results may not be clinically relevant. |
| 7. *Physician notified of unsatisfactory specimen but results ordered anyway* | Isolate(s) reported may or may not be the etiologic agent of the patient's disease.<br><br>Specimen unsatisfactory for processing. Results may or may not be significant.<br><br>Specimen unsatisfactory for processing. Results reported on physician's orders. |
| 8. *Contaminated sputum* | Specimen consists primarily of oropharyngeal material; please submit another specimen. |

In lieu of speciation or workup

*Obvious frank pathogens should be reported; i.e., "*B. fragilis* plus mixed_____ flora" or "Beta-lactamase positive anaerobic gram-negative rod plus mixed ____ flora."

| Condition | Sample Addendum to Bacteriology Report |
|---|---|
| 9. *Incomplete speciation is warranted* | If complete speciation, typing or grouping of this isolate is clinically indicated, please consult Microbiology.<br><br>This specimen was received in a condition inappropriate for optimum production of clinically useful information. Collection of another specimen is suggested. |

**When controls reveal a test to be potentially inaccurate, the results of that test cannot be reported. Remember that a SPECIMEN can be "out of control."**

## BODY FLUIDS

**Amniotic fluid** – fluid produced by the innermost layer of the placenta early in gestation and contained within the amniotic sac surrounding the embryo in utero.

**Ascitic fluid** – serous fluid aspirated from the abdominal cavity (the peritoneum).

**Bile** – a brown-green fluid secreted by the liver and either poured into the intestine or concentrated in the gallbladder.

**Bone marrow** – the soft, highly cellular, blood-forming tissue that fills bone cavities.

**Joint (synovial) fluid** – alkaline, thick fluid contained in joint cavities, bursae, and tendon sheaths serving as a lubricant.

**Pericardial fluid** – fluid contained within the membranous sac that encases the heart.

**Peritoneal fluid** – same as ascitic fluid.

**Pleural fluid** – fluid within the thoracic cavity which encases the lungs.

**Spinal fluid** – fluid contained within the membranous coverings of the spinal cord and brain within the space known as the subarachnoid space.

**Transudate** – fluid which has passed through a membrane or extruded from a tissue and characterized by its low viscosity, lack of protein, and cells or cellular debris, and by having a specific gravity under 1.013.

## EYE

**Conjunctiva** – the mucous membrane covering the anterior surface of the eyeball and the under surfaces of the eyelids.

**Inner canthus** – the inner (nasal) angle formed by the union of the upper and lower eyelids.

**Lid** – folds of skin that protect the anterior eyeball surface.

## GENITAL (FEMALE)

**Bartholin's gland** – one of two, small, mucous-secreting glands on either side of the vaginal orifice.

**Cervical aspirate** – mechanical withdrawal of material from the cervix.

**Culdocentesis** – aspiration of fluid from recto-uterine excavation by puncture of the vaginal wall.

**Endocervical** – from the interior of the cervix.

**Endometrium** – the mucous membrane comprising the inner lining of the uterine cavity.

**Fallopian tube** – tube from uterus to ovary.

**Female genital** – nondescript term generally taken to mean a vaginal/cervical specimen.

**Lochia** – the final vaginal discharge occurring 1-2 weeks after childbirth.

**Ovary** – reproductive egg-forming gland within the pelvis in the female, lying lateral to the uterus.

**Perineum** – the space between the anus and the scrotum of the male and the anus and vulva of the female.

**Placenta** – highly vascularized organ of pregnancy, composed of multiple layers within the gravid uterus, supplying nutrients and gas exchange to the fetus.

**Placenta (C-section)** – (see placenta) a result of a cesarean section.

**Urethral** – from the membraneous canal conveying urine from the bladder to the exterior of the body.

**Uterus** – hollow, muscular organ in the female in which the fetus develops.

**Vaginal** – from the canal that extends from the vulva to the cervix.

**Vulva** – the region of the external female genitals.

GENITAL (MALE)

**Lesion** – a more or less circumscribed pathologic or traumatic injury to tissue.

**Penile exudate** – exudate expressed through the urethra.

**Prostate** – a gland which, in the male, surrounds the neck of the bladder and the urethra.

**Urethral** – see above.

LOWER RESPIRATORY TRACT

**Bronchial** – referring to the large air passages which dichotomously branch within the lungs.

**Bronchial aspirate** – material collected from the bronchi by means of instrumentation.

**Fiberoptic** – collection of material with an instrument designed for visualization of the lower respiratory area and for specimen collection.

**Sputum** – matter ejected from the lungs, bronchi, and trachea through the mouth.

**Tracheal** – the tube from the larynx to the bronchi.

**Transtracheal aspirate** – material obtained by surgical passage of a catheter through the tracheal wall and into the lower respiratory area.

UPPER RESPIRATORY TRACT

**Ear** – unless specified, refers to the external ear.

**Mouth and dental** – gums, gingivae, teeth, root canals, tongue, etc.

**Nasopharynx** – that part of the pharynx above the soft palate.

**Nose (nasal)** – in microbiology the term usually refers to culture obtained from about 1-2 cm deep within the nostril.

**Sinus** – any body cavity, hollow space, or open channel.

**Throat** – that area within the deep oral cavity between and including the tonsillar pillars from where cultures are obtained.

**STOOL/RECTAL** – a term referring to the fecal discharge from the bowels.

SURFACE SPECIMENS

**Burn** – traumatic lesion caused by contact of tissue with heat.

**Cyst** – any liquid- or exudate-containing sac.

**Decubitus** – ulceration due to prolonged pressure of lying down or sitting.

**Exudate** – fluid containing protein, cells or solid material which has escaped from blood vessels as a result of injury or inflammation.

**Laceration** – a cut.

**Lesion** – see GENITAL (MALE).

**Paronychia** – inflammation involving the folds of skin around the fingernails.

**Skin** – external body covering.

**Stoma** – any small opening or orifice on a free surface, i.e. the opening from a colostomy or ileostomy site.

**Suture** – a surgical "stitch."

**Ulcer** – a loss of integrity of a cutaneous or mucous surface lining resulting from the sudden or gradual sloughing of necrotic tissue.

**Vesicle** – a small blister containing a serous liquid.

SURGICAL SPECIMENS

**Abscess** – localized collection of pus in a cavity formed by disintegration of tissue.

**Aspirate** – removal of fluids from a cavity by suction.

**Biopsy** – surgical removal of small portions of tissue from a living body for the purpose of establishing a precise diagnosis.

**Bone** – mineralized connective tissue that makes up the skeleton of vertebrates.

**Clot** – a semisolid mass, usually of blood or lymph.

**Drain** – an artifically placed device used to create a channel by which fluid or pus can be exited from a cystic space or body cavity.

**Exudate** – see SURFACE SPECIMENS.

**Fistula** – an abnormal passage or communication between two organs or to the outside.

**Hematoma** – a tumor of effused blood - a bruise.

**IV catheter** – tubing used to infuse sterile material into the veins.

**Prosthesis** – an artificial body part.

**Pus** – a liquid inflammatory product of leukocytes and fluid.

**Stone** – a very hard mass or calculus usually composed of mineral salts.

**Tissue** – a surgically removed mass of body cells.

**Wound** – see lesion, GENITAL (MALE).

URINE

**Catheterized** – urine aspirated from a urinary catheter.

**Midstream** – urine collected in a container after the first few milliliters of urine has been passed.

**Suprapubic** – urine surgically aspirated with syringe and needle by direct puncture into the bladder.

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